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# Design of a Novel, Multi-port-addressable Bioaerosol Collection System<sup>1,2</sup>

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**Trina Vian**  
**MIT Lincoln Laboratory**  
**tvian@ll.mit.edu**

<sup>1</sup>This work was sponsored by the Department of the Air Force under Air Force Contract #F19628-00-C-0002. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the United States Government.

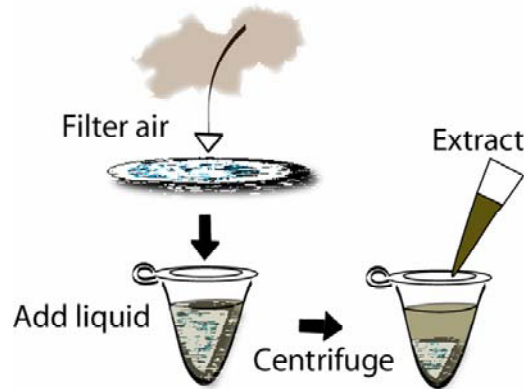
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# Difficulties with Current Samplers

## Filter-based Sampling



### Limitations:

- Target organisms desiccated during collection
- Suboptimal recovery efficiency
- Labor-intensive post-collection processing

## Other Methods



### Limitations:

- Sample still needs to be transferred
- Suboptimal recovery efficiency
- Possibly labor-intensive post-collection processing

## Viable Sampling



### Limitations:

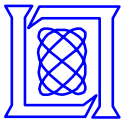
- Short sampling duration
- Culture results may take days to weeks
- Growth media selection bias



# Air Collection System Requirements

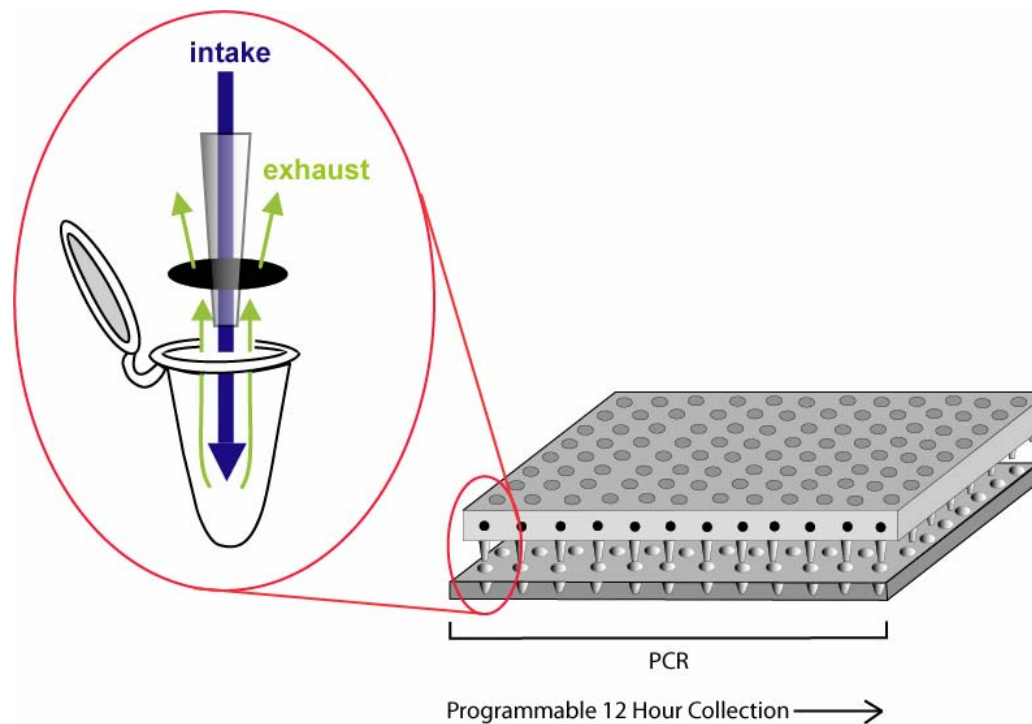
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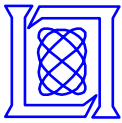
- **High collection and extraction efficiency in the 1 – 10 micron particle-size range**
- **A robust collection scheme that offers reconfigurable sample collections at any time interval from nominally 3 to 12 hours**
- **A collection scheme that preserves viability, for even fragile vegetative cells and viruses**
- **A self-sealing mechanism that renders the collected samples safe for retrieval, handling and transport**
- **A collection format optimized for compatibility with standard assays such as PCR while reducing subsequent laboratory processing requirements**



# Aerosol Collector Concept

- **Use impaction collection into coated 96-well PCR plates**
  - PCR plate can be directly assayed at processing laboratory
  - Sample does not require elution, sample preparation or transfer
  - Mineral oil coating enhances capture efficiency, maintains sample viability and serves as an evaporation barrier for PCR
  - Time resolution of sample is achieved through serial activation of the rows of the plate

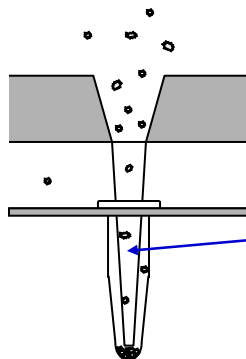
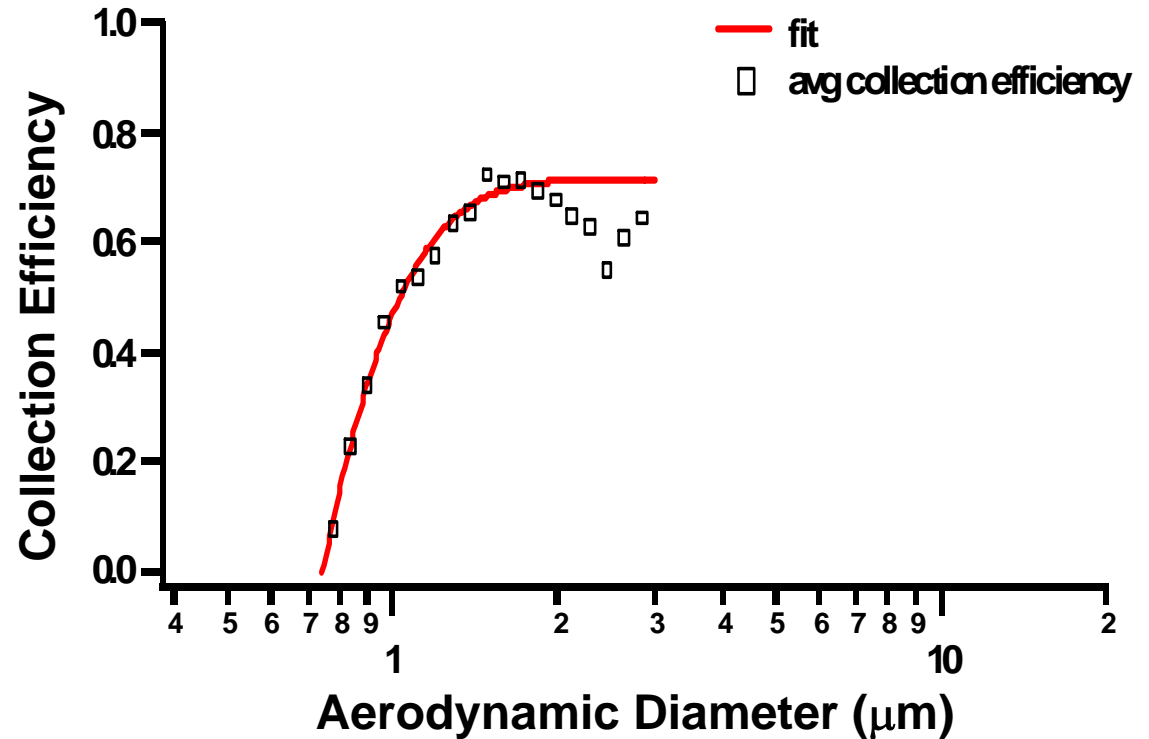




# Impaction into Uncoated PCR Tubes

## Design features

- > 50% collection/extraction efficiency for 1 - 10  $\mu\text{m}$  particles based on particle counts and bio-assays
- no sample transfer required (particles immobilized in reaction well)
- expect mineral-oil coating to increase collection efficiency



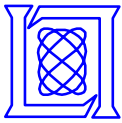
Impaction nozzle protrudes deep into the PCR tube



# Preliminary Design Parameters

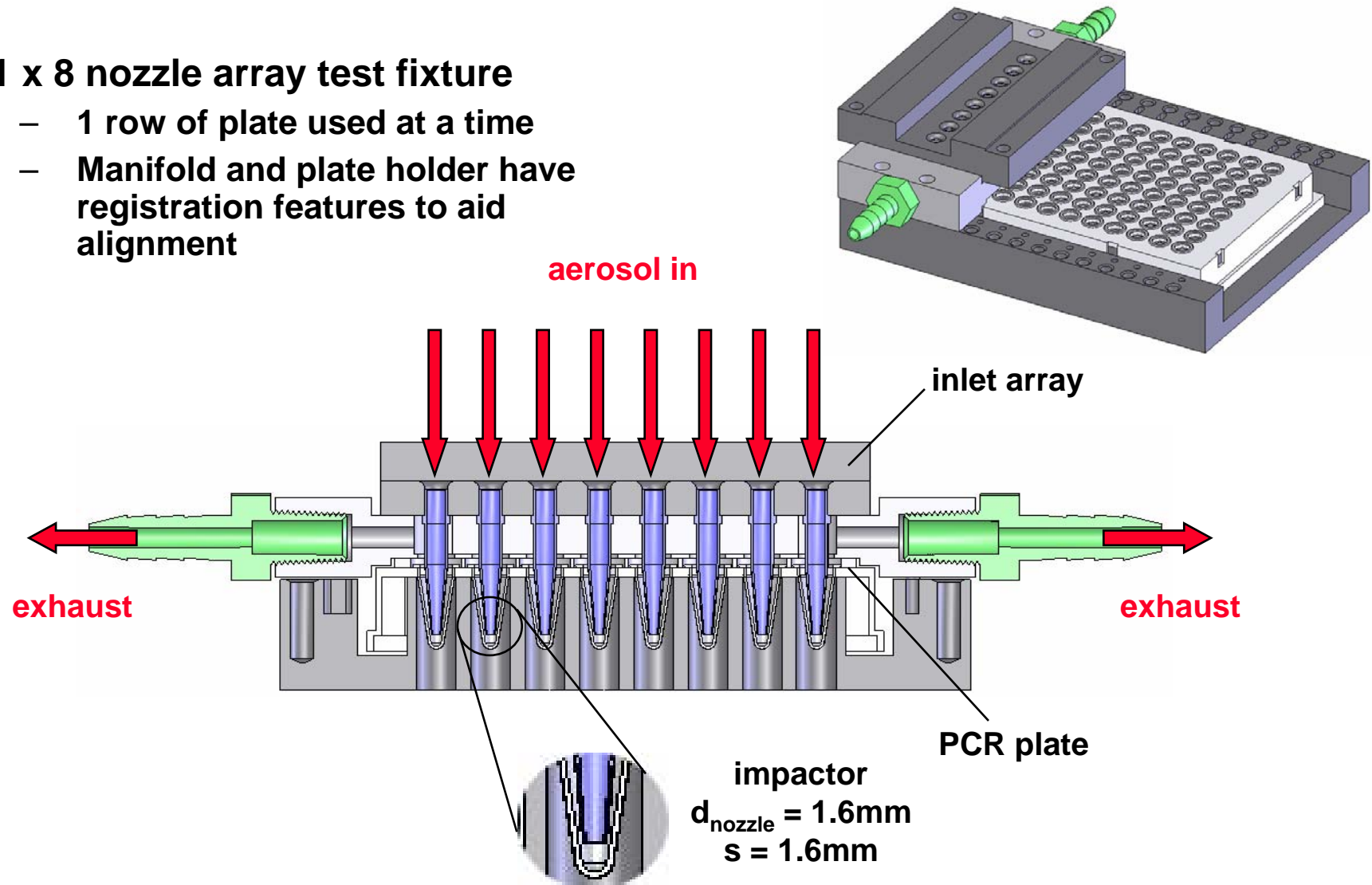
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- **Aerosol Inlet Design**
  - Disperse particles evenly over 1 x 12 impaction manifold
- **Flow Rate for Impaction Nozzles**
  - Design for flow rate of 3 lpm/nozzle
  - Collection efficiency greater than 50%
- **Sealing Mechanism**
  - Integrate plate sealer into overall design
  - Facilitate ease of handling and transport for the operator
- **Communication and Control**
  - Remotely readable and modifiable user parameters such as sampling interval, sampler location and system status
  - Valve actuation by controller



# PCR Plate Impaction Test Fixture

- 1 x 8 nozzle array test fixture
  - 1 row of plate used at a time
  - Manifold and plate holder have registration features to aid alignment

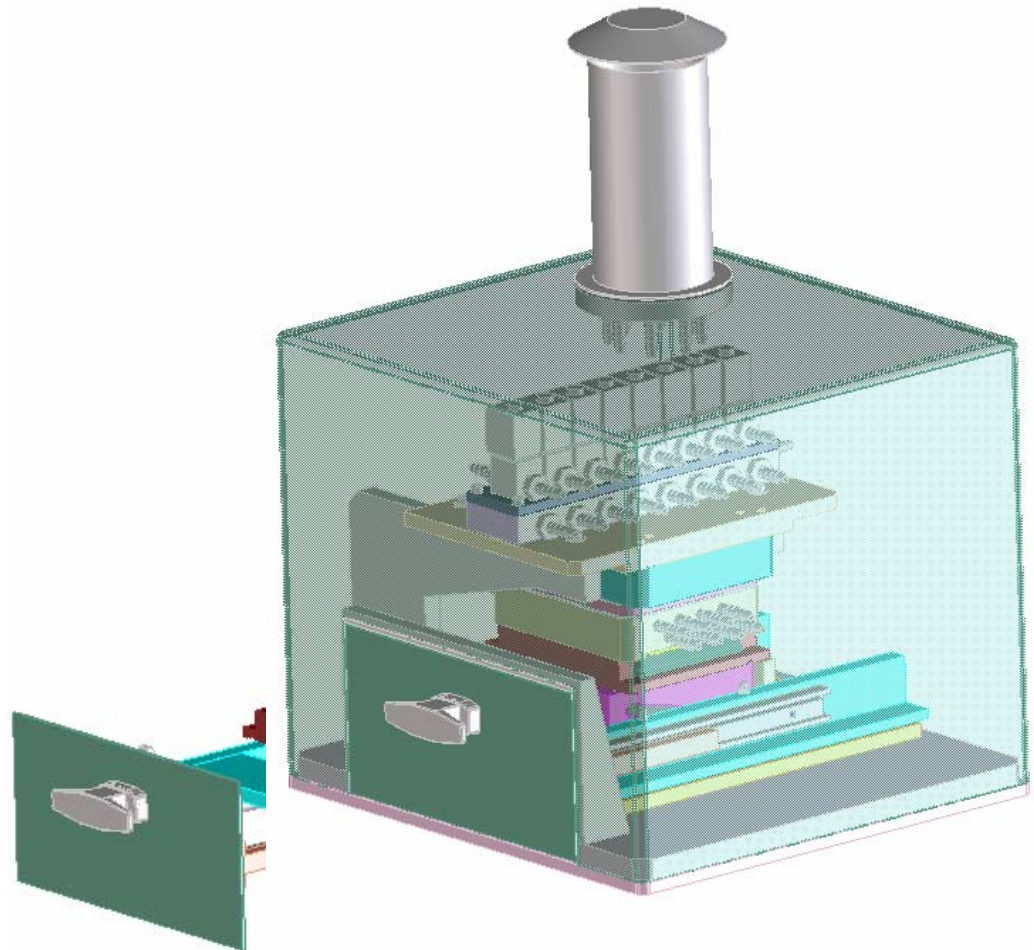




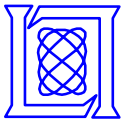


# Notional System Design

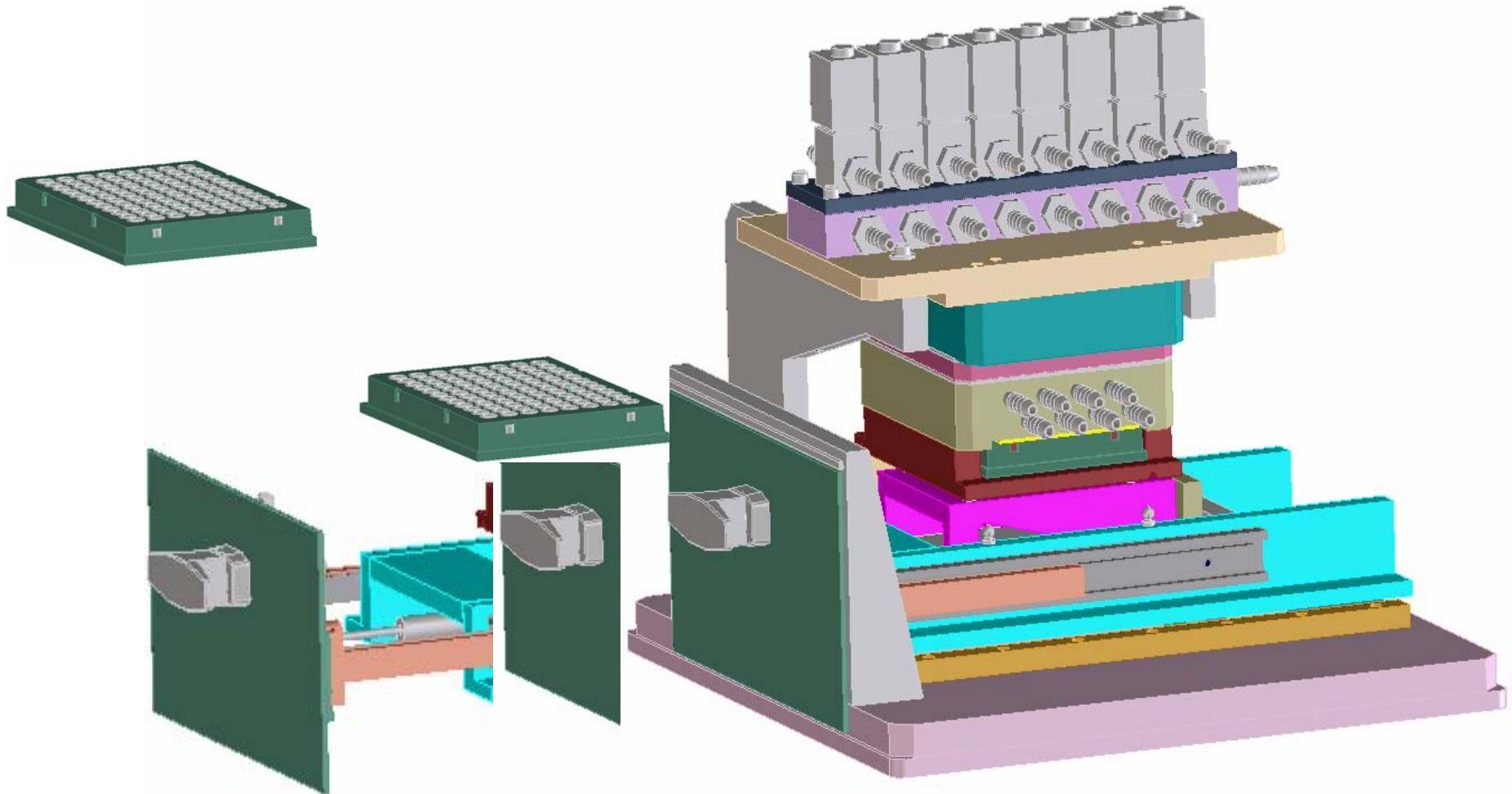
- **Air collector subsystems\***
  - Inlet stack
  - Impaction nozzle manifold (8 x 12 array)
  - PCR plate and receptacle
  - Valve bank
  - Exhaust manifold
  - Pump (not shown)
  - Viable sampler (not shown)
- **Mechanical subsystems**
  - Drawer assembly
    - Drawer, plate shuttle, pneumatic spring, cam, lock
  - Valve bank
- **Electrical**
  - Communication and control
    - User interface, pump and valve switching, power supplies
  - Environmental conditioning (not shown)
- **Plate Sealing**
  - Adhesive film roller (not shown)



\*All tubing omitted for clarity



# Plate Loading

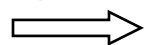




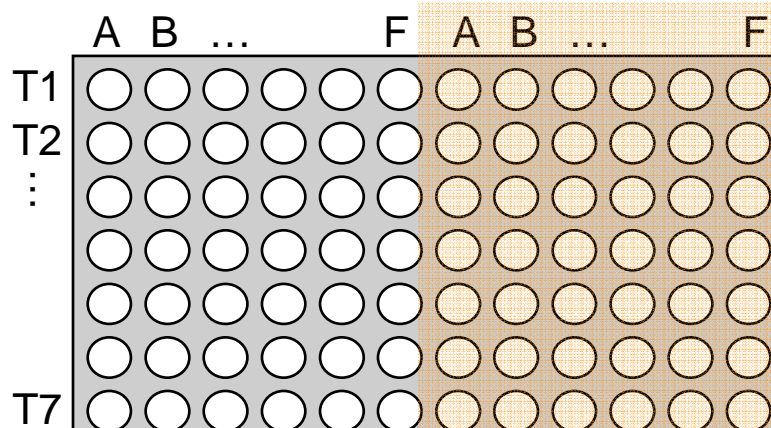
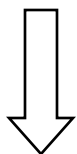
# Processing and Interpretation Benefits

## Example PCR Plate Layout

Agent tested: A, B, etc



Time-resolved  
air collects into  
each well



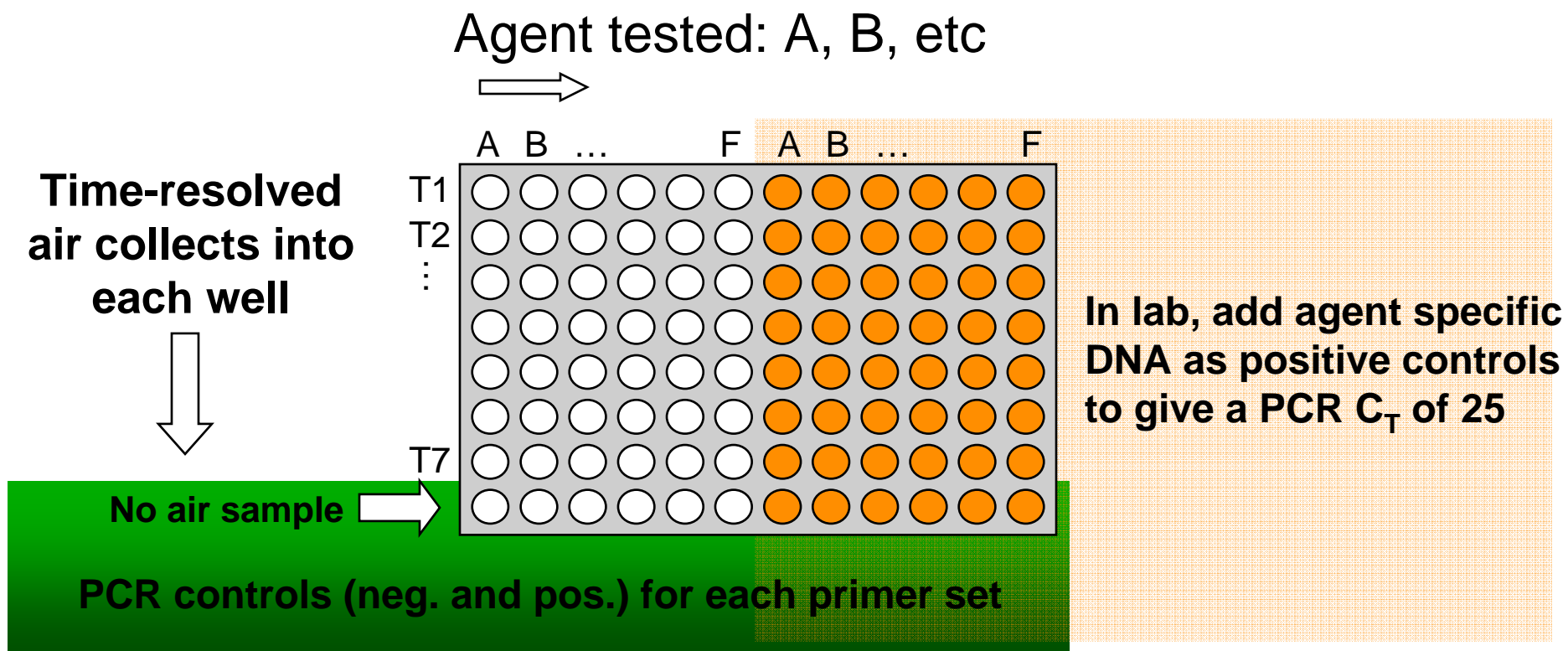
In lab, add agent specific  
DNA as positive controls  
to give a PCR  $C_T$  of 25

No air sample

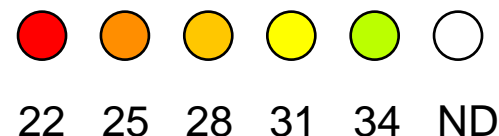
PCR controls (neg. and pos.) for each primer set

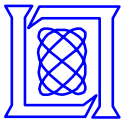


# Standard PCR Reaction – No Agent Present

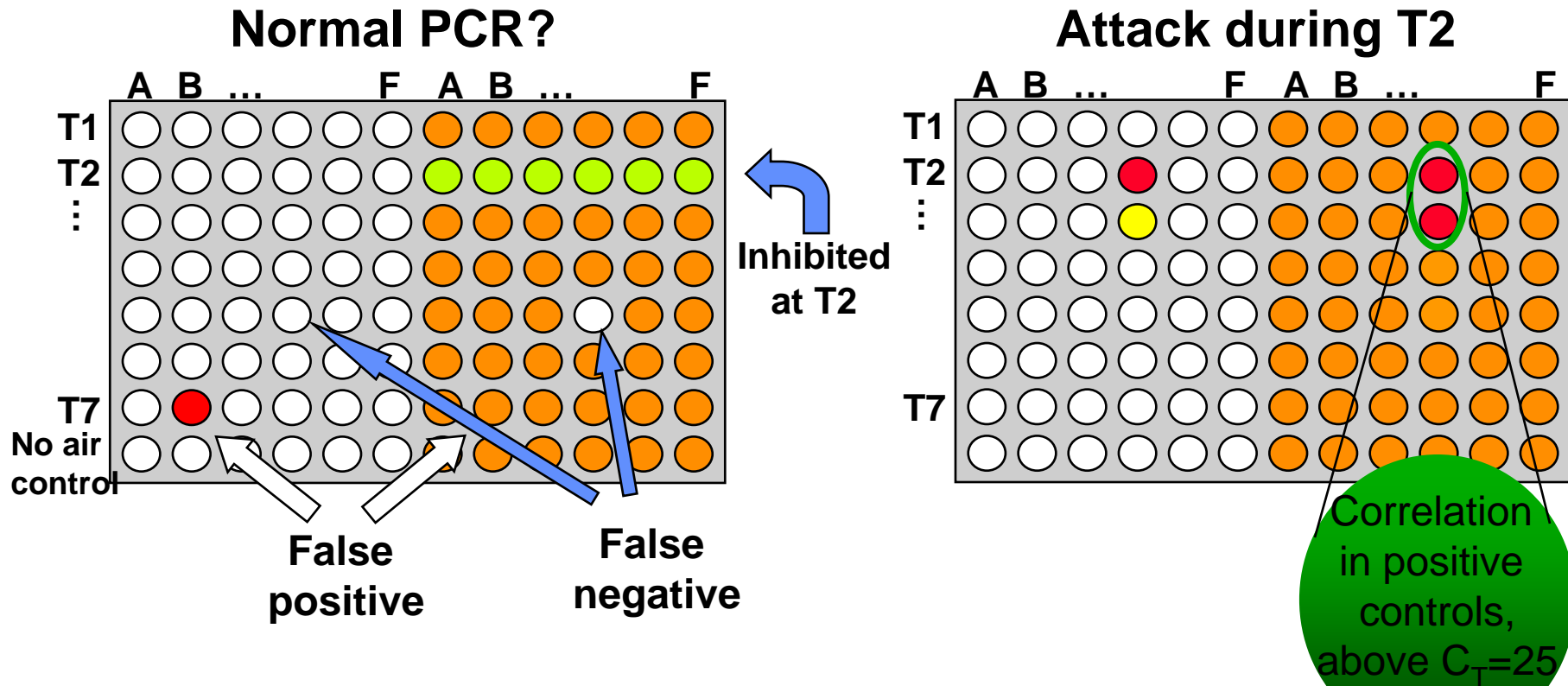


PCR Cycle Threshold Key



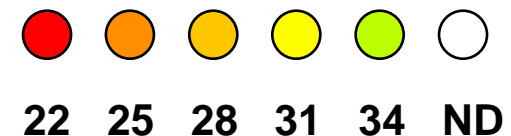


# Other Possibilities and Interpretations



- Daily knowledge of system performance
- Effect of inhibitors (with time resolution)
- False positive and negative rates
- Confirmation of true positives in control wells
- Simple plate layout for lab-based error reduction

## PCR Cycle Threshold Key





# Summary

High collection and extraction efficiency in the 1 – 10 micron particle-size range

$\epsilon_{\text{collection}} > 50\%$   
 $\epsilon_{\text{extraction}} \sim 100\%$

A robust collection scheme that offers reconfigurable sample collections at any time interval from ~ 3 to 12 hours

Timed valving allows for user-defined collection interval

A collection scheme that preserves viability, for even fragile vegetative cells

Mineral oil coated impaction substrate keeps organisms viable

A self-sealing mechanism that renders the collected samples safe for handling, retrieval and transport

Film seal isolates sample

A collection format optimized for compatibility with PCR and designed to minimize the subsequent laboratory processing requirements

PCR plate usage allows for easy pipetting of reagents



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# Collector Close Up

